Extra Chapter

**Chapter 1**

***Objective:***

***Methods:***

*Completed Work:* Over 350 bumble bees were collected in northern Vermont from 13 different field sites during the summer of 2014. The bees were netted randomly while foraging on flowers. Queens and males were caught as well as workers. The bees were put on dry ice in the field and were transferred to a -80oC freezer within 12 hours of being captured. At each site, bee abundance and vegetation surveys were performed on 100m transects. In addition, forging honeybees were netted and pollinator friendly flowers collected at each site. GPS coordinates, elevation, weather conditions, and nearest town were also logged at each location.

In order to assay each bee for *Nosema*, the ventriculus was dissected from the bee by pulling on the last segment (terga) of the abdomen. The ventriculus for each bee was then homogenized in 500uL of GITC buffer with a polypropylene pestle for one minute. These were then vortexed and 10uL of the homogenized bee gut were put into each chamber of a hemocytometer. Counts were made of the *Nosema* spores present using a traditional Neubauer® counting grid and the two chambers were averaged together resulting in a total count.

The beta and gamma terms were derived from the empirical data of the survey. Beta is the conversion rate from susceptible to infected. Gamma is the conversion rate from infected to critically infected (diseased state). Beta=(#infected/total#), gamma=(#critically infected/#infected). The death rates (muA and muB), fecal deposition rates (alpha1 and alpha2) were estimated, as was the initial rate of infection (I1).

This model describes the dynamics in this parasite system. Parameters were calculated or estimated depending on availability of data to describe the dynamics of the infection rate. Terms were developed to describe the system. The system of equations that makes up the model is shown below:

dS =−SPβ−SμA dt

dI1 =SPβ−I1μA−I1γ dt

dI2 =I1Pγ−I2μB dt

dP =I1α1+I2α2−Pθ dt

The equations were solved using an R package, “deSolve”. Vectors for initial values and parameters were created and a time sequence of 150 days was set up to replicate the period that bumble bees are active in a temperate climate. The infected and critically infected vectors were subtracted from 1 to represent the susceptible population.

*Remaining Work:*

***Preliminary Findings:*** The prevalence of *Nosema* was found to be 20.2% across all species and castes. The critically infected bees comprised of 5% of all infected bees. There was found to be variability among species. *B. vagans* showed the lowest susceptibility to *Nosema* while *B. borealis* and *B. ternarius* showed the highest (Fig 2). However, this variability was found to be insignificant using a contingency table with a Chi-squared test (p=0.299) and we failed to reject the null hypothesis that prevalence across species is the same. When looking at prevalence by caste, we found that the percent infected was highest in males and lowest in workers contrary to our hypothesis. However, the sample size of males was significantly lower than the other two castes (Fig. 3).

The differences were also found to be insignificant. The results of a Chi-squared test yielded a p-value of 0.468. Proximity to honeybees was also found to be statistically insignificant. Though prevalence was higher in bumble bees caught near honeybee apiaries, the Chi-squared test showed that the difference was minimal (p=0.481) (Fig 4). As these three factors appeared to show little effect upon the prevalence of *Nosema*, a more simplified model of this system (ignoring caste and species effects on infection) could be created using the total prevalence of the infected bees and total prevalence of critically infected bees as rates in an elementary SIR-style model.